

WHAT IS CLAIMED IS:

1 1. A method of inhibiting expression of an endogenous cellular gene
 2 in a cell, the method comprising the step of:
 3 contacting a first target site in the endogenous cellular gene with a first
 4 zinc finger protein, wherein the K_d of the zinc finger protein is less than about 25 nM;
 5 thereby inhibiting expression of the endogenous cellular gene by, at least
 6 about 20%.

1 2. The method of claim 1, wherein the step of contacting further
 2 comprises contacting a second target site in the endogenous cellular gene with a second
 3 zinc finger protein.

1 3. The method of claim 2, wherein the first and second target sites are
 2 adjacent.

1 4. The method of claim 3, wherein the first and second zinc finger
 2 proteins are covalently linked.

1 5. The method of claim 1, wherein the first zinc finger protein is a
 2 fusion protein comprising a regulatory domain.

1 6. The method of claim 5, wherein the first zinc finger protein is a
 2 fusion protein comprising at least two regulatory domains.

1 7. The method of claim 2, wherein the first and second zinc finger
 2 proteins are fusion proteins, each comprising a regulatory domain.

1 8. The method of claim 7, wherein the first and second zinc finger
 2 protein are fusion proteins, each comprising at least two regulatory domains.

1 9. A method of inhibiting expression of an endogenous cellular gene
 2 in a cell, the method comprising the step of:
 3 contacting a target site in the endogenous cellular gene with a fusion zinc
 4 finger protein comprising six fingers and a regulatory domain, wherein the K_d of the zinc
 5 finger protein is less than about 25 nM;

0509744-070201

1 10. The method of claim 1, wherein the cell is selected from the group
2 consisting of animal cell, a plant cell, a bacterial cell, a protozoal cell, or a fungal cell.

12. The method of claim 11, wherein the cell is a human cell.

1 ~~subpg 8~~ 14. The method of claim 1, wherein the endogenous cellular gene is a
2 selected from the group consisting of VEGF, ER α , IGF-I, c-myc, c-myb, ICAM, and
3 Her2/Neu.

1 16. The method of claim 1, wherein the inhibition of gene expression
2 prevents gene activation.

1 18. The method of claim 1, wherein the method further comprises the
2 step of first administering to the cell a delivery vehicle comprising the zinc finger protein,
3 wherein the delivery vehicle comprises a liposome or a membrane translocation
4 polypeptide.

1 19. The method of claim 1, wherein the zinc finger protein is encoded
2 by a zinc finger protein nucleic acid operably linked to a promoter, and wherein the
3 method further comprises the step of first administering the nucleic acid to the cell in a
4 lipid:nucleic acid complex or as naked nucleic acid.

Sub
A10³

~~of claim~~

Sub
A10³

Sub
A10³

Sub
A10³

Sub
A10³

Sub
A10³

Sub
A10³

Sub
All

Sub
All

Sub
All

1 31. A method of activating expression of an endogenous cellular gene,
 2 the method comprising the step of:
 3 contacting a first target site in the endogenous cellular gene with a first
 4 zinc finger protein, wherein the K_d of the zinc finger protein is less than about 25 nM;
 5 thereby activating expression of the endogenous cellular gene to at least
 6 about 150%.

1 32. The method of claim 31, wherein the step of contacting further-
 2 comprises contacting a second target site in the endogenous cellular gene with a second
 3 zinc finger protein.

1 33. The method of claim 32, wherein the first and second target sites
 2 are adjacent.

1 34. The method of claim 33, wherein the first and second zinc finger
 2 proteins are covalently linked.

1 35. The method of claim 31, wherein the first zinc finger protein is a
 2 fusion protein comprising a regulatory domain.

1 36. The method of claim 35, wherein the first zinc finger protein is a
 2 fusion protein comprising at least two regulatory domains.

1 37. The method of claim 32, wherein the first and second zinc finger
 2 proteins are fusion proteins, each comprising a regulatory domain.

1 38. The method of claim 37, wherein the first and the second zinc
 2 finger protein are fusion proteins, each comprising at least two regulatory domains.

1 39. A method of activating expression of an endogenous cellular gene,
 2 the method comprising the step of:
 3 contacting a target site in the endogenous cellular gene with a fusion zinc
 4 finger protein comprising six fingers and a regulatory domain, wherein the K_d of the zinc
 5 finger protein is less than about 25 nM;
 6 thereby activating expression of the endogenous cellular gene to at least
 7 about 150%.

1 40. The method of claim 31, wherein the cell is selected from the
2 group consisting of an animal cell, a plant cell, a bacterial cell, a protozoal cell, or a
3 fungal cell.

1 41. The method of claim 40, wherein the cell is a mammalian cell.

1 42. The method of claim 41, wherein the cell is a human cell

1 43. The method of claim 31, wherein expression of the endogenous
2 cellular gene is activated to at least about 200-500%.

1 ~~44. The method of claim 31, wherein the endogenous cellular gene is a~~
2 ~~selected from the group consisting of FAD2-1, EPO, GM-CSF, GDNF, VEGF, and LDL-~~
3 ~~R.~~

1 45. The method of claim 31, wherein the endogenous cellular gene is
2 VEGF.

1 46. The method of claim 31, wherein the activation of gene expression
2 prevents repression of gene expression.

1 47. The method of claim 35 or 37, wherein the regulatory domain is
2 selected from the group consisting of a transcriptional activator, or a histone
3 acetyltransferase.

1 ~~48. The method of claim 31, wherein the method further comprises the~~
2 ~~step of first administering to the cell a delivery vehicle comprising the zinc finger protein,~~
3 ~~wherein the delivery vehicle comprises a liposome or a membrane translocation~~
4 ~~polypeptide.~~

1 49. The method of claim 31, wherein the zinc finger protein is encoded
2 by a zinc finger protein nucleic acid operably linked to a promoter, and wherein the
3 method further comprises the step of first administering the nucleic acid to the cell in a
4 lipid:nucleic acid complex or as naked nucleic acid.

1 50. The method of claim 31, wherein the zinc finger protein is encoded
2 by an expression vector comprising a zinc finger protein nucleic acid operably linked to a

09897844-070201

SUB
P16

3 promoter, and wherein the method further comprises the step of first administering the
4 expression vector to the cell.

1 51. The method of claim 50, wherein the expression vector is a viral
2 expression vector.

1 52. The method of claim 50, wherein the expression vector is a
2 retroviral expression vector, an adenoviral vector, a DNA plasmid vector, or an AAV
3 expression vector.

1 53. The method of claim 50, wherein the zinc finger protein is encoded
2 by a nucleic acid operably linked to an inducible promoter.

1 54. The method of claim 50, wherein the zinc finger protein is encoded
2 by a nucleic acid operably linked to a weak promoter.

1 55. The method of claim 31, wherein the cell comprises less than about
2 1.5×10^6 copies of the zinc finger protein.

1 56. The method of claim 31, wherein the target site is upstream of a
2 transcription initiation site of the endogenous cellular gene.

1 57. The method of claim 31, wherein the target site is adjacent to a
2 transcription initiation site of the endogenous cellular gene.

1 58. The method of claim 31, wherein the target site is adjacent to an
2 RNA polymerase pause site downstream of a transcription initiation site of the
3 endogenous cellular gene.

1 59. The method of claim 31, wherein the zinc finger protein comprises
2 an SP-1 backbone.

1 60. The method of claim 59, wherein the zinc finger protein comprises
2 a regulatory domain and is humanized.

1 61. A method of modulating expression of an endogenous cellular gene
2 in a cell, the method comprising the step of:

SUB
A17

09897844-070201
T02020-448680

SUB
A18

SUB
A19

3 contacting a first target site in the endogenous cellular gene with a first
4 zinc finger protein;
5 thereby modulating expression of the endogenous cellular gene.

1 62. The method of claim 61, wherein the step of contacting further
2 comprises contacting a second target site in the endogenous cellular gene with a second
3 zinc finger protein.

1 63. The method of claim 62, wherein the first and second target sites
2 are adjacent.

1 *SUB A20* 64. The method of claim 63, wherein the first and second zinc finger
2 proteins are covalently linked.

1 65. The method of claim 61, wherein the first zinc finger protein is a
2 fusion protein comprising a regulatory domain.

1 66. The method of claim 65, wherein the first zinc finger protein is a
2 fusion protein comprising at least two regulatory domains.

1 67. The method of claim 62, wherein the first and second zinc finger
2 proteins are fusion proteins, each comprising a regulatory domain.

1 68. The method of claim 67, wherein the first and second zinc finger
2 protein are fusion proteins, each comprising at least two regulatory domains.

1 69. A method of modulating expression of an endogenous cellular gene
in a cell, the method comprising the step of:

4 contacting a target site in the endogenous cellular gene with a fusion zinc
5 finger protein comprising six fingers and a regulatory domain;
thereby modulating expression of the endogenous cellular gene.

1 70. The method of claim 61, wherein the cell is selected from the
2 group consisting of animal cell, a plant cell, a bacterial cell, a protozoal cell, or a fungal
3 cell.

1 71. The method of claim 70, wherein the cell is a mammalian cell

0989744-070001

SUB A213

1 72. The method of claim 71, wherein the cell is a human cell.

1 73. ~~The method of claim 61, wherein the endogenous cellular gene is a~~
 selected from the group consisting of VEGF, ER α , IGF-I, c-myc, c-myb, ICAM,
 Her2/Neu, FAD2-1, EPO, GM-CSF, GDNF, and LDL-R.

1 74. The method of claim 61, wherein the endogenous cellular gene is
 2 VEGF.

1 75. The method of claim 65 or 67, wherein the regulatory domain is
 2 selected from the group consisting of a transcriptional repressor, a transcriptional
 3 activator, an endonuclease, a methyl transferase, a histone acetyltransferase, and a histone
 4 deacetylase.

1 76. ~~The method of claim 61, wherein the method further comprises the~~
 2 ~~step of first administering to the cell a delivery vehicle comprising the zinc finger protein,~~
 3 ~~wherein the delivery vehicle comprises a liposome or a membrane translocation~~
 4 ~~polypeptide.~~

1 77. ~~The method of claim 61, wherein the zinc finger protein is encoded~~
 2 ~~by a zinc finger protein nucleic acid operably linked to a promoter, and wherein the~~
 3 ~~method further comprises the step of first administering the nucleic acid to the cell in a~~
 4 ~~lipid:nucleic acid complex or as naked nucleic acid.~~

1 78. The method of claim 61, wherein the zinc finger protein is encoded
 2 by an expression vector comprising a zinc finger protein nucleic acid operably linked to a
 3 promoter, and wherein the method further comprises the step of first administering the
 4 expression vector to the cell.

1 79. ~~The method of claim 78, wherein the expression vector is a viral~~
 2 ~~expression vector.~~

1 80. The method of claim 78, wherein the expression vector is a
 2 retroviral expression vector, an adenoviral expression vector, a DNA plasmid expression
 3 vector, or an AAV expression vector.

1 81. The method of claim 78, wherein the zinc finger protein is encoded
2 by a nucleic acid operably linked to an inducible promoter.

1 82. The method of claim 78, wherein the zinc finger protein is encoded
2 by a nucleic acid operably linked to a weak promoter.

1 83. The method of claim 61, wherein the cell comprises less than about
2 1.5×10^6 copies of the zinc finger protein.

1 84. The method of claim 61, wherein the target site is upstream of a
2 transcription initiation site of the endogenous cellular gene.

1 85. The method of claim 61, wherein the target site is adjacent to a
2 transcription initiation site of the endogenous cellular gene.

1 86. The method of claim 61, wherein the target site is adjacent to an
2 RNA polymerase pause site downstream of a transcription initiation site of the
endogenous cellular gene.

1 87. The method of claim 61, wherein the zinc finger protein comprises
2 an SP-1 backbone.

1 88. The method of claim 88, wherein the zinc finger protein comprises
2 a regulatory domain and is humanized.

0997844 02020" 111876860